Fund "Nauka" Project № 18025 Resume – Competitive-based Session 2018:
"Establishment of an infrastructure for 3D- and bioprinting of personalized spatially controlled implants, prostheses, medical devices and consumables for research, diagnostic and therapeutic purposes in MU-Varna"
Project leader: Assoc. prof. Stoyan Pavlov Pavlov, MD, PhD

The 3D printers are class of CNC machines, which are manufacturing objects via additive methods, in contrast to the more "traditional" subtractive manufacturing. The materials are added precisely, one layer at a time until the object is entirely constructed. The main methods of 3D printing include extrusion-based 3D printing, FDM (Fused Deposition Modeling), SLS (Selective Laser Sintering), SLA (Stereolithography), MJF (Multi Jet Fusion), DLP (Digital Light Processing), etc.

The methods themselves and the range of materials for each of them are quite different, but the 3D printing process is the same for all:

- One layer of the object is built, then on top of it starts the construction of the next layer.
- In order to build overhanging structures, removable support structures are used
- After the 3D printing, the object is post-processed (via sanding, painting, polishing, nanocoating, etc.), and then the construct is done.

The resulting object is not as strong and resilient as the injection-molded or subtractive milled objects. However, the possibility to model complex structures is a significant advantage of additive 3D manufacturing, especially in the field of biomedicine.

The 3D bioprinting uses natural or synthetic biopolymers, which are extruded on a building platform. When printed, they generate a three-dimensional network of fibrous structures, which gives structural integrity to the object. In composites of two or more types of biopolymers the compounds intersect in a complex structure similar to the extracellular matrix in the multicellular organisms.

The properties of the biopolymers can be further augmented with additives such as growth factors (ILGF, VEGF, BMP, FGF etc.) or high-performance materials (graphene, metals, carbon, nano silicates etc.).

The three-dimensional cell cultures resemble the natural conditions in the tissues and demonstrate better the behavior of the cell types, important for the regenerative medicine (embryonal and adult stem and progenitor cells, induced pluripotent stem cells, pericytes etc.). The most promising bio-inks are hydrophilic composite structures, known as hydrogels. Their high viscosity and water content provide excellent conditions for the accuracy of the 3D printing, the structural integrity of the construct, and the suitability of the material to sustain and promote cell adhesion, proliferation, and differentiation.

For the overhang issues, related to the 3D printing of soft biological material, especially good sacrificial supporting material is the gelatin and the name of the technology is FRESH method (Freeform reversible embedding of suspended hydrogels). This technology allows the development of advanced bioengineering constructs, which can be subsequently populated with different types of stem cells.

Finally yet importantly, all surgical therapies can benefit from diagnostic 3D-printed models of organs constructed from diagnostic medical images of the patients. They serve as training models to simulate and plan the operation in advance and thus reduce the risks for the patient. This approach significantly decreases the intraoperative time, the complication rate, and the recovery time of the patient. The anatomy teaching can also benefit a lot from the 3D-printed objects.

Since 2018, the Medical University "Prof Dr. Paraskev Stoyanov"- Varna by an initiative of the Department of Anatomy and cell biology started building a 3D manufacturing laboratory for the creation of 3D devices for educational, research, diagnostic and therapeutic purposes. In fulfillment of this project, the Medical University of Varna acquired a modular 3D printer - Hyrel 3D Hydra 16 A, equipped with the following modular heads:

1. Modular heads for fused deposition modeling (FDM) 3D printing of thermoplastics: Those plastics can be used as Anatomical 3D models, diagnostic and training surgical models, laboratory equipment, casings for electronics, and bioreactors et cetera.

- ❖ MK1-250 Standard extruder for FDM 3D printing. It provides support over filament-based thermo-polymers with melting temperatures up to 250°C like PLA, ABS, PETG, ASA, Nylon, et cetera.
- ❖ MK2-250 Dedicated extruder for elastic materials like TPU, TPE, various elastomers, and silicones.
- ❖ MK1-450 Dedicated extruder for high-performance engineering plastics with melting temperature up to 450°C like the PEAK family (PEEK, PEK, PEKEK et cetera), PEI, composites et cetera.

2. Modular heads for extrusion-based deposition 3D printing: Those modular heads support a broad range of gels and solutions, including hydrogels, clays, pastes, emulsions, silicones, wax, metal clays (including precious metal clays) et cetera. The current extruder works on ambient temperature, with 10ml sterile syringes

equipped with Luer lock needles. It can be further upgraded with the same type of deposition system for various temperatures and syringe sizes.
3. 100x microscope modular head for quality control.
4. The machine can be further upgraded with various modular heads for several subtractive and additive CNC technologies.

The 3D printer will be used in several ongoing PhD projects, including:

1. “Ankle stability after talar substitution with personalized 3D printed prosthesis”; Department of Orthopedy and traumatology; Dr Preslav Penev;
2. “Bioprinting and morphological analysis of a 3D scaffold for biosynthetic implants”; Department of Anatomy and cell biology; Dr Petar Valchanov;
3. “Bioprinting for the development of microfluidic systems (organ-on-chip) for pathophysiological and pharmacological studies of non-alcoholic fatty liver disease and accompanying multisystemic metabolic disorders”; Department of Physiology and pathophysiology; Assoc. Prof Kamelia Bratoeva, PhD;
4. “Generation of personalized bioproducts for the management of the frontal ocular surface”; Department of Ophthalmology and visual sciences; Dr. Dimiter Grupchev.

Results:

1. Acquired tangible and intangible assets:
❖ Multifunctional open system for three-dimensional printing Hydra 16AT, Hyrel 3D, USA;
❖ Provided SDS10 extruder, purchased under Project № 19027 “Bioprinting and morphological analysis of a 3D scaffold for biosynthetic implants”.
2. Repairs and construction carried out (if applicable):
❖ Re-equipment of hall 220 of the Department of Anatomy and Cell Biology in order to accept the 3D Printer.
3. Raised funds as a result of cooperation with Bulgarian and foreign universities, research institutions, etc .:
❖ “Physical breast anthropomorphic models and technology for their production (PHENOMENO)”; project leader: assoc. prof. eng. Kristina Bliznakova – funded by grant № 101008020 for activities “Marie Skłodowska-Curie” of the European Union Research and Innovation Program “Horizon 2020”;
❖ “ARPHA: Development of anthropomorphic radiological phantoms” – funded by the National Research Fund, 2021-2023.
4. Perspectives and opportunities for future development of the research activity in MU - Varna:

The built infrastructure provides methodological support for several current projects:

- “Ankle stability after talar substitution with 3D individually printed prosthesis”; Department: Orthopedics and Traumatology; Preslav Plamenov Penev, MD, PhD;
- “Bioprinting and morphological analysis of 3D matrix for biosynthetic implants”; Department: Anatomy and Cell Biology; Petar Stamatov Valchanov, MD, PhD;
- “Bioprinting for the construction of microfluidic systems (organ on chip) for pathophysiological and pharmacological studies of non-alcoholic fatty liver disease and concomitant multisystemic metabolic disorders”; Department: Physiology and Pathophysiology, assoc. prof. Kamelia Zhechkova Bratoeva, MD, PhD.

Apart from the methodological support that the infrastructure provides for the mentioned current projects, the built infrastructure is also key for the participation in two new projects:

1. “Physical breast anthropomorphic models and technology for their production (PHENOMENO)”; project leader: assoc. prof. eng. Kristina Bliznakova – funded by grant № 101008020 for activities “Marie Skłodowska-Curie” of the Research and Innovation Program of the European Union “Horizon 2020”;
2. “ARPHA: Development of anthropomorphic radiological phantoms” – funded by the National Research Fund, 2021-2023.